

Gametophytes of *Equisetum diffusum*

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Equisetum gametophytes have been studied for many years (Buchtien, 1887; Hauke, 1967, 1968, 1969, 1971, 1977; Duckett, 1970, 1972, 1973, 1977; and literature cited in these references). The gametophytes of all *Equisetum* species except *E. diffusum* Don have been described. In a recent monograph of *Equisetum* subg. *Equisetum* (Hauke, 1979), I presented an evolutionary sequence of species arranged according to gametophyte specialization which did not parallel the sequence based on sporophyte specialization. Subsequently I was able to obtain viable spores of *E. diffusum* and to culture them. The purpose of this paper is to describe gametophytes of *Equisetum diffusum* and to discuss their evolutionary implications.

MATERIALS AND METHODS

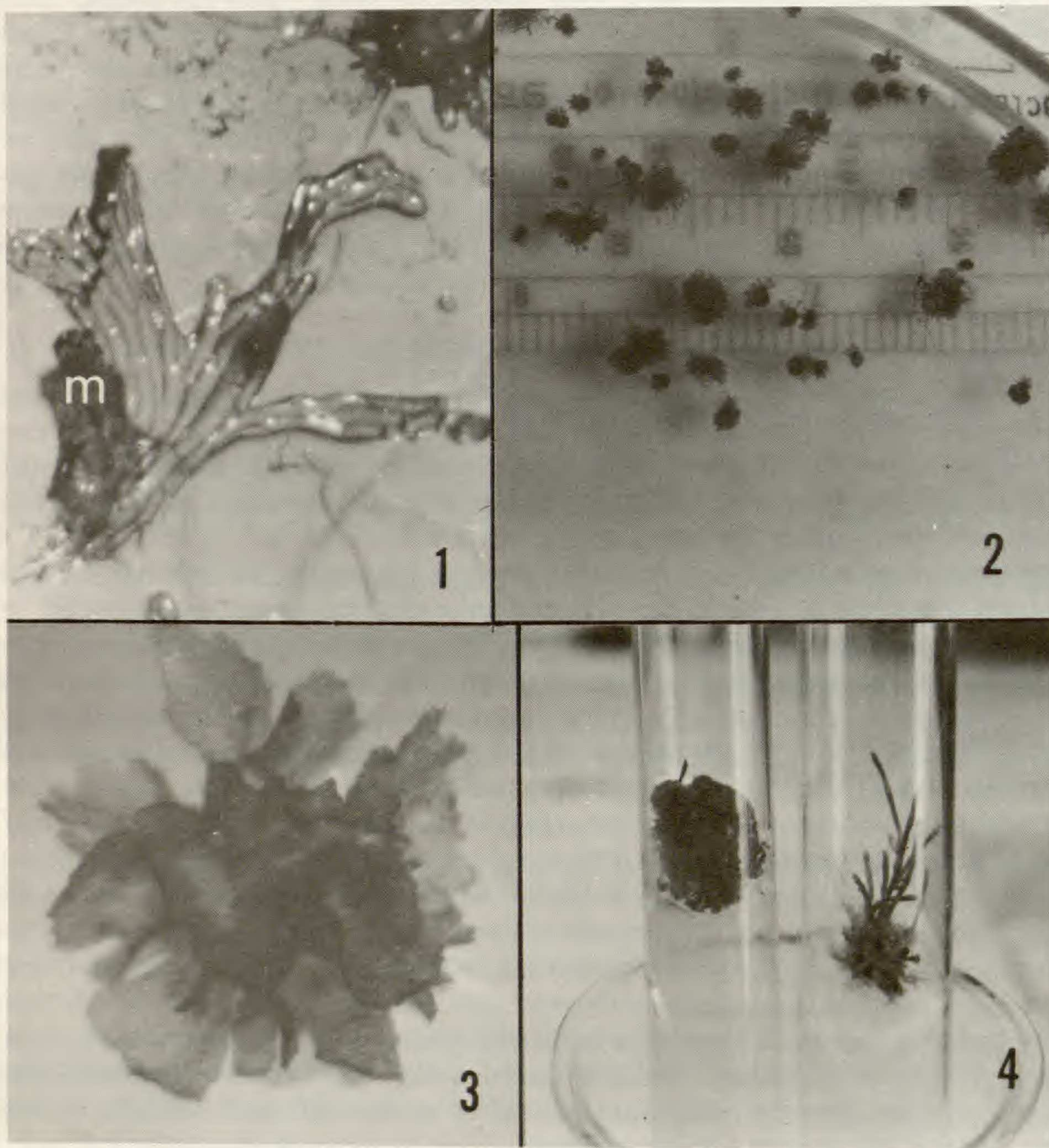
On 24 February 1974, I collected living rhizomes of *E. diffusum* along the road between Chail and Kandaghat, near Simla, Himachal Pradesh state, India. These flourished in pots in the greenhouse at the University of Rhode Island. In August 1978, I first noticed cones developing on the ends of unbranched, new stems in two pots. The plants in these pots apparently had died back and regrown. The first cone was removed before it expanded, surface sterilized with 50% commercial sodium hypochlorite bleach, rinsed with sterile distilled water, and dissected in sterile distilled water. Ten drops of the spore suspension were inoculated onto petri dishes containing Bold's Basal Medium (BBM) in 1.5% agar. Subsequent cones were allowed to open naturally, and the spores were shaken onto the surface of the solidified nutrient medium. The culture dishes were placed in a growth chamber on a 12 hr light/dark cycle at a temperature of 20/15°C under 40 watt cool white fluorescent tubes yielding 8000 ergs/cm²/sec radiant energy at the surface of the cultures, as measured with a YSI radiometer.

Gametophytes were grown in isolation to determine crossability, self-compatibility, and the possible occurrence of apogamy. Spores that had begun to germinate in petri dishes were transferred aseptically into 15 mm diameter test tube slants of BBM agar capped with metal caps and placed in racks for incubation in the growth chamber. Initially the isolated gametophytes grew more slowly than those left in petri dishes, possibly because the test tube caps shaded the gametophytes; when the tubes were positioned to allow full light intensity on the agar surface, the growth rate accelerated.

RESULTS

The spores of *E. diffusum*, like those of all other species of *Equisetum*, are spherical, chlorophyllous, thin-walled, alete, and have two hygroscopic elaters attached at their middles, which form four strap-like arms with spoon-shaped tips. Under suitable conditions of moisture, temperature, and light, they germinate readily in 1–2 days by dividing eccentrically to produce a small rhizoid cell that

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FIGS. 1–4. Gametophytes of *Equisetum diffusum*. FIG. 1. Gametophyte 24 days old showing rhizoids, meristem (m), and plates, $\times 70$. FIG. 2. Antheridial and archegonial gametophytes 64 days old, $\times 1.8$. FIG. 3. Archegonial gametophyte 50 days old, showing broad plates with irregular margins, $\times 30$. FIG. 4. Gametophytes 158 days old with sporophytes. Left = selfed bisexual gametophyte, sporophyte visible 20 days earlier. Right = crossed female gametophyte, sporophyte visible 55 days earlier. Note smaller size of gametophyte on right, $\times 1$.

loses its chloroplasts and a large, green somatic cell. Further division of the latter produces a flattened, linear gametophyte which branches to form several plates of cells. Eventually a parenchymatous cushion bearing plates dorsally and rhizoids ventrally is established by a marginal meristem. (Fig. 1).

Sex organs begin to appear 35 days after inoculation on the basal cushion meristem of initially unisexual gametophytes. Young male and female gametophytes look alike, but with continued growth they become dimorphic (Fig. 2).

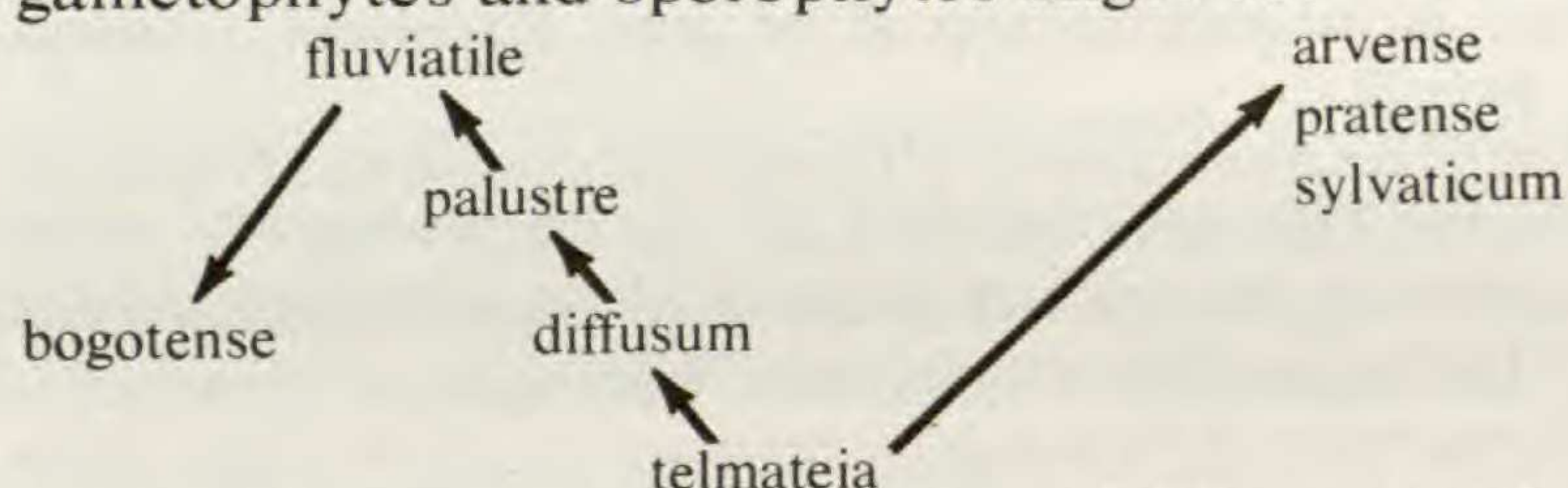
The female gametophytes become larger than the male, develop numerous plates, and assume a grass-green color (Fig. 3). The plates of *E. diffusum* are up to 2 mm broad and have irregular margins and thickened bases. Archegonia develop at the base of the plates and consist of three tiers of four neck cells each. The egg is embedded in the cushion. The terminal neck cells elongate to four times longer than broad and spread apart in an arching manner. Seen from above, the spread terminal neck cells resemble a pinwheel.

The male gametophytes remain smaller than the female, with only sparse plate development, and are yellowish to pinkish. Antheridia develop from the cushion and protrude somewhat at maturity, becoming twice as long as wide. They discharge sperm by two or four cap cells. The cap cells in this species are not so distinctive as in other species and apparently may divide anticlinally, so that at times there are three or five cap cells.

Female gametophytes that are not fertilized within a certain time begin to produce antheridia. The marginal meristem that has been producing archegonia grows out into an antheridial lobe. In petri dish cultures, where interaction between gametophytes is possible, the bisexual condition may become apparent within 50 days. In isolation tubes, where interaction is not possible, bisexuality is delayed to 90 days or later. Some isolated gametophytes 150 days old still appear only female. Gametophytes apparently cease growth when they begin bearing sporophytes (Fig. 4). If they are unisexual at that time, they never become bisexual. Unlike the situation usually seen in other pteridophytes, *Equisetum* gametophytes normally bear several sporophytes per gametophyte (Fig. 4).

Whereas more than 50% of the gametophytes in plate cultures are antheridial, in isolation tubes, in the absence of interaction between gametophytes (but presumably with better nutrition), only 5 out of 42 (12%) were male. The other 37 were female. One tube inadvertently received two gametophytes initially, one of which became male and the other female. Three of the male gametophytes were transferred to tubes with female gametophytes and flooded. The tube with two gametophytes was flooded (Fig. 4), as were 17 tubes with only female gametophytes. The four tubes with both male and female gametophytes all showed sporophytes a month later. None of those with only female gametophytes did. The tubes originally containing only female gametophytes were reflooded, and eventually they became bisexual and selfed (Fig. 4). At 158 days post inoculation the experiment was terminated and the tubes refrigerated to stop growth. Later they were examined and discarded, at which time 14 of the 17 selfed gametophytes had visible sporophytes. In two cases, the sporophytes all looked achlorophyllous.

That sequence, it is true, placed *E. diffusum* and *E. telmateia* side by side, but it also considered *E. bogotense* to be most like *E. diffusum*. This is the greatest discrepancy between the schemes based on sporophyte and gametophyte evolution. Perhaps there is no reason why the two generations should be correlated evolutionarily, since they presumably evolve for different environmental fitness, but it seems appropriate to consider the whole plant in a single evolutionary scheme. In that case, one might expect the gametophyte to reflect more conservative traits, as the reproductive structures of other plants are assumed to do. For an example of independent selection for floral and vegetative fitness in flowering plants, however, see Wilken (1978). If taxonomy is intended to be phylogenetic, should it emphasize gametophyte or sporophyte? One factor which diminishes the usefulness of the gametophytic stage in pteridophyte taxonomy is the paucity of characters it possesses. A compromise scheme, utilizing characters of both gametophytes and sporophytes might be:



It injures my sense of the fitness of things to look at two species with sporophytes as similar as those of *E. bogotense* and *E. diffusum* and to separate them widely in a classification. Yet the gametophyte as well as the sporophyte must be considered in arriving at any taxonomy which claims to be phylogenetic. In fact, if it is more conservative in evolution, then it should be given greater emphasis in taxonomy.

Isolation experiments showed that the gametophytes of *E. diffusum* outcross readily. They also self readily in most cases, but the absence of any detectable sporophytes on three of 17 selfed gametophytes, and the chlorotic sporophytes on two others, indicates some lethal load (see Lloyd, 1974). The absence of sporophytes on individuals that were not flooded indicates the absence of apogamy in *E. diffusum*. Ease of selfing and absence of apogamy are also found in the other species of *Equisetum*.

I wish to thank Dr. Stoddard Malarky for helping me collect *E. diffusum* and Dr. Roger Goos for reading the manuscript.

ADDENDUM

In a paper which appeared while this article was awaiting publication, Duckett (1979) made several observations which are pertinent here. He reported that prolonged culture and numerous attempted fertilizations were required to obtain maximal sporophyte frequencies, and suspected "leaky lethals," but on the basis of crossing and selfing tests discounted that possibility. He noted that the initiation of sporophytes is accompanied by cessation of gametophyte growth, and attributed this to allelopathic substances from the sporophyte. He reported that polyembryony is present in all species, but is uncommon and occurs mostly in

species with rapid sex change from female to bisexual, or with numerous archegonial lobes. I have observed polyembryony to be common in isolated, selfed gametophytes not only of *E. diffusum*, but also of *E. fluviatile*, *E. hyemale* var. *affine*, and *E. arvense*, and suspect that they were all larger and therefore with more receptive archegonia when flooded than were those of Duckett's experiments.

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